REMARKS

Interview Summary

The Applicant and the Applicant's Representative thank the Examiner for the personal interview conducted with the Examiner at the U.S. Patent and Trademark Office on 21 August 2007. The Applicant and the Applicant's Representative greatly appreciate the Examiner's time, interest, and consideration. The Examiner prepared an Interview Summary dated 21 August 2007 which the Applicant accepts as an accurate summary of the substance of the interview. Much of the content of the interview will also be addressed in this Amendment and Response in greater detail.

Substance of Interview

During the Interview on 21 August 2007, the Applicant explained how the claimed invention is fundamentally distinct from the prior art. The Examiner has generally relied on the combination of (a) a prior art colony forming assay and (b) an ATP bioluminescence assay to provide the basis for an obviousness rejection under 35 U.S.C. § 103(a). The Applicant explained that the claimed invention is not merely a novel combination of elements found in the prior art. The claimed invention is, in fact, fundamentally different from any of the cited art, either alone or in combination.

Proliferation is the process by which cells increase in number by division to produce daughter cells identical to the parent cell. Differentiation is a process by which unspecialized cells develop the structures and functions characteristic of a particular mature cell type. In the case of stem cells, a stem cell can proliferate to form additional stem cells. Alternatively, a stem cell can further differentiate into committed cells and differentiated cells.

The traditional colony forming assay (CFA) found in the prior art is a differentiation assay which requires manual counting of differentiated colonies, generally

after 14 days in culture. The cells are allowed to differentiate and form colonies of functionally mature cells so they can then be identified morphologically according to colony type and counted manually. In contrast, the claimed invention is directed to a proliferation assay. The claimed invention provides a high throughput assay method for rapidly determining the proliferative status of a population of cells. While proliferation and differentiation are related, they are fundamentally different processes. Proliferation is required for the process of differentiation to occur, but differentiation is not required for the process of proliferation to occur. To the Applicant's knowledge, the claimed invention provides the first method which allows determination of the proliferative status of a population of cells and the first method which allows the process of proliferation to be separated out from the process of differentiation.

These issues will be addressed in greater detail in the context of the pending rejections and the cited prior art references.

Claim Amendments

After entry of the present amendment, claims 1-4, 6-15, 18-28, 31, 42-44, and 57-60 are currently pending in the application. Claims 1, 6, 7, 28, and 57 have been amended. Claim 5 has been cancelled. Claims 59 and 60 are new. Support for all claim amendments is found in the specification as filed, and there is no new matter presented in any of the claim amendments.

The recitation of transferrin in claim 1 has been deleted. Support for this amendment is found throughout the specification and specifically in original claim 1 in the application as filed. The original claims as filed did not include transferrin.

As the Examiner suggested in the Interview on 21 August 2007, the step of contacting the primitive hematopoietic cell population with a proliferation agent has been introduced into claim 1. Amended claim 1 reads, in relevant part, "contacting the primitive hematopoietic cell population with a proliferation agent, the proliferation agent selected from the group consisting of a single growth factor, a mix of growth factors, a

single cytokine, a mix of cytokines, and combinations thereof." Support for contacting the primitive hematopoietic cell population with one or more cytokines and/or growth factors is found throughout the application as originally filed, and specifically in claims 5 and 28 of the originally filed application. Numerous specific examples of the use of one or more cytokines and/or growth factors are provided at page 21, lines 5-19; page 27, line 10 - page 29, line 24; Example 3, page 37, and Table 1, page 38.

The term "proliferation agent," as used in amended claim 1, is defined as "a single growth factor, a mix of growth factors, a single cytokine, a mix of cytokines, and combinations thereof." The term "proliferation agent" was used in claim 5 as amended in the Amendment and Response dated 12 February 2007, without objection by the Examiner in her subsequent Office Action dated 19 March 2007. Additional support for use of the term "proliferation agent" appears throughout the application and specifically on page 14, lines 12-27 of the application as filed. Additional examples of one or more cytokines and/or growth factors use as a "proliferation agent" include the following:

> Yet another aspect of the present invention is a high-throughput assay method for rapidly identifying a **compound capable of modulating the proliferative status** of a population of primitive hematopoietic cells. Application as filed, p.8, lines 18-20 (emphasis added).

> The terms "modulating the proliferative status" or "**modulating the proliferation**" as used herein refers to the **ability of a compound to alter the proliferation rate** of a population of hematopoietic stem or progenitor cells. A compound may be toxic wherein the proliferation of the cells is slowed or halted, or the **proliferation may be enhanced** such as, for example, **by the addition to the cells of a cytokine or growth factor**. Application as filed, page 12, line 26 - page 13, line 1 (emphasis added).

> Such **proliferation enhancing compounds** include, for example, **cytokines and growth factors**.
>
> Application as filed, page 12, lines 8-9, (emphasis added).

Applicant believes that the term "a proliferation agent, the proliferation agent selected from the group consisting of a single growth factor, a mix of growth factors, a single cytokine, a mix of cytokines, and combinations thereof" is adequately supported by the application as filed.

Claims 6, 7, and 28 have been amended to change their dependency. Each of claims 6, 7, and 28 had depended from claim 5. The limitations of claim 5 have been introduced into amended claim 1, and claim 5 has been canceled. Therefore, claims 6, 7, and 28 have been amended to depend from claim 1 in order to provide proper antecedent basis.

Claim 57 has been amended to change its dependency in order to provide proper antecedent basis for the recitation of transferrin which no longer appears in claim 1.

New claims 59 and 60 have been added. New claims 59 and 60 recite the element of transferrin which has been deleted from amended claim 1. Support for this amendment is found on page 19, lines 26-27; on page 34, lines 17-18; and on page 35, lines 4-6 of the application as filed.

All claim amendments are fully supported in the application as filed, and no new matter has been introduced in any of the claim amendments. Applicant also notes that no additional search is required due to the claim amendments, as all limitations present in the currently pending claims as amended have already been searched.

Withdrawn Rejections

Applicant thanks the Examiner for withdrawing the rejection of the pending claims under 35 U.S.C. § 103(a) as being unpatentable over Crouch et al., *Journal of Immunological Methods*, 160: 81-88 (1993), in view of Bell et al., U.S. Patent

Application Publication No. US 2002/0120098 A1, and further in view of Moore, U.S. Patent No. 5,328,844.

Claim Rejections

All pending claims stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Crouch et al., *Journal of Immunological Methods*, 160: 81-88 (1993), in view of Bell et al., U.S. Patent Application Publication No. US 2002/0120098 A1, and further in view of Bauer et al., U.S. Patent No. 6,440,407. Applicant respectfully submits that none of the cited references, either alone or in combination, render the claimed invention obvious. Applicant requests that the Examiner withdraw these rejections in view of the remarks presented herein.

As stated in the MPEP:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). (MPEP 706.02(j), emphasis added.)

Applicants submit that the Examiner's rejection of the pending claims is improper because the Examiner has failed to provide a clear suggestion or motivation that would have led one of ordinary skill in the art to combine or to modify the prior art references in the manner suggested by the Examiner. More importantly, even if the prior art references were combined as the Examiner suggests, the combination would not result in the claimed invention because the combined references do not teach or suggest all of the claim limitations as required for a proper 35 U.S.C. § 103(a) rejection.

The claimed invention is directed to a method for a high-throughput assay for determining the **proliferative status** of a population of primitive hematopoietic cells. In simple terms, the Examiner relies on (1) Crouch et al. for teaching an ATP bioluminescence assay, (2) Bell et al. for teaching various tissue culture conditions, and (3) Bauer et al. for disclosing the use of transferrin in cell growth media. The Examiner asserts that:

> It would have been obvious to one of ordinary skill in the art at the time of the instant invention to substitute the culture growth medium as taught by Bell as modified by Bauer for expanding and stimulating proliferation of progenitor hematopoietic cells and maintaining the cells, so as to be tested for ATP bioluminescence assay as in the method of Crouch in order to determine their proliferative status.
> (Office Action, mailed 29 March 2007, p.6.)

As amended, claim 1 recites:

> 1. A high-throughput assay method for rapidly **determining the proliferative status of a population of primitive hematopoietic cells**, the method comprising the steps of:
>
> (a) incubating a cell population comprising primitive hematopoietic cells in a cell growth medium comprising fetal bovine serum having a concentration of between 0% and about 30%, methyl cellulose having a concentration of between about 0.4% and about 0.7%, and in an atmosphere having between about 3.5% oxygen and about 7.5% oxygen;
>
> (b) contacting the primitive hematopoietic cell population with a proliferation agent, the proliferation agent selected from the group consisting of a single growth factor, a mix of growth factors, a single cytokine, a mix of cytokines, and combinations thereof;

(c) contacting the cell population with a reagent capable of generating luminescence in the presence of ATP; and

(d) detecting luminescence generated by the reagent contacting the cell population, the level of luminescence indicating the amount of ATP in the cell population, wherein the amount of ATP **indicates the proliferative status of the primitive hematopoietic cells**.

None of the prior art references teach or suggest a method for determining the proliferative status of a population of primitive hematopoietic cells as recited in the pending claims. Accordingly, the Applicant submits that the pending claims are patentable over all art cited and of record.

Crouch et al.

The Examiner asserts that:

> "Crouch et al. disclose an assay method for determining the proliferative status (cell proliferation) of a population of primitive (lymphoblastic, promyelocytic) hematopoietic cells. The hematopoietic cells are granulocyte-macrophage colony-forming cells (GM-CFC) and granulocyte colony-forming cells (G-CFC), i.e. TF-1 and NSF-60 cells."
> (Office Action, mailed 29 March 2007, p.3.)

The Applicant respectfully disagrees with the Examiner's understanding of Crouch et al. Crouch et al. do not, in fact, disclose an assay method for determining the proliferative status of primitive hematopoietic cells. Crouch et al. do not even disclose the use of any primitive hematopoietic cells for any purpose. Applicant suggests that the confusion may have arisen because Crouch et al. discusses the use of granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating

factor (G-CSF). Perhaps the Examiner may have confused the growth factors used in Crouch et al. with cell types that are not disclosed in Crouch et al.

The cells that are used in Crouch et al. are not primitive hematopoietic cells. On the contrary, the cell types to which the Examiner refers (TF-1 and NFS-60 cells) are actually transformed cell lines, not primitive hematopoietic cells. Unlike primitive hematopoietic cells, transformed cell lines are in a state unregulated growth in culture, much like an *in vitro* tumor. According to the American Type Culture Collection (ATCC, at www.atcc.org), the TF-1 cell line was derived from an erythroblast from a human patient with erythroleukemia. The ATCC comments further state that "[t]he TF-1 cell line was established by T. Kitamura, et al. in October 1987 from a heparinized bone marrow aspiration sample from a 35 year old Japanese male with severe pancytopenia." Also according to the ATCC, the NFS-60 cell line was derived from a lymphoblast from a mouse with myelogenous leukemia. The ATCC comments further state that "[t]he M-NFS-60 cell line was derived from a myelogenous leukemia induced with the Cas-Br-MuLV wild mouse ecotropic retrovirus."

While Crouch et al. does disclose an ATP bioluminescence assay, Crouch et al. does not teach or suggest an assay for determining the proliferative status of a population of primitive hematopoietic cells. Nor does Crouch et al. teach or suggest the claimed cell growth medium.

Bell et al.

The Examiner asserts that:

> "Bell et al. teach a cell growth medium comprising 30 % fetal bovine serum and about 0.4 % to about 0.7 % (0.8 %) methyl cellulose in an atmosphere having between about 3.5 % to 7.5 % (5%) [oxygen] for use in culturing the hematopoietic cells."
> (Office Action, mailed 29 March 2007, p.5.)

Applicant assumes that Examiner inadvertently left out the word "oxygen" in referring to the teaching of Bell et al. The Examiner further asserts that Bell discloses a "cell proliferation assay," and the Examiner specifically cites page 9 paragraphs [0084] - [0087], and Examples 1 and 2 of Bell et al. (Office Action, mailed 29 March 2007, p.5.) Contrary to the Examiner's assertion, however, Bell et al. utilizes the colony forming assay of the prior art and does not disclose a cell proliferation assay as the Examiner suggests.

One of the passages of Bell et al. cited by the Examiner provides:

> [0084] The Detection of Erythroid Progenitors in the **Colony Formation Assay**
>
> [0085] Hemoglobin enhances the growth of erythroid progenitors, notably the BFU-E progenitor population as shown by a **well-known technique** in the field of erythropoiesis research. This technique, referred to as the **colony formation assay (CFA)**, is the most widely used biological assay to identify and enumerate erythroid progenitors present in hematopoietic tissue such as blood and bone marrow. Cell populations containing erythroid progenitors are plated in semi-solid suspensions of methyl cellulose, agar, low melting agarose or related substances in nutrient culture medium containing 1-3 U/ml Epo and 10 ng/ml IL-3 to specifically stimulate erythroid progenitors. **Plates are incubated at 37° C. for 14 days during which time the erythroid progenitors form characteristic hemoglobinized (red) colonies**. **Two distinct but related erythroid progenitors can be distinguished based on colony size and morphology**: BFU-E, the most primitive recognizable erythroid progenitor forms large multilobular colonies whereas the more mature CFU-E forms smaller spherical colonies. Under these conditions other

> myeloid progenitors, notably the colony forming unit--granulocyte-macrophage (CFU-GM), also form morphologically identifiable non-hemoglobinized (white) colonies which are readily distinguished from erythroid colonies. Additional cytokines, notably IL-1, IL6, stem cell factor, flt-3 ligand, and granulocyte-macrophage colony stimulating factor, may be included in the CFA to stimulate both erythroid and nonerythroid progenitor proliferation.
> Bell et al., page 9, paragraphs [0084] - [0085], emphasis added.

This passage from Bell et al. makes it clear that the traditional colony forming assay of the prior art was used by Bell et al. It is also clear that the colony forming assay used by Bell et al. is a differentiation assay. In the procedure used by Bell et al., the plates must be incubated for 14 days in order for the cells to differentiate into mature cell types. After the cells have differentiated, the colonies are identifiable based on the morphology of the differentiated cell type.

The Examiner also cites Examples 1 and 2 of Bell et al. as disclosing a "cell proliferation assay." Examples 1 and 2 of Bell et al., however, also disclose only a colony forming assay, or differentiation assay. In relevant part, Examples 1 and 2 of Bell et al. state the following:

> The LDMNC were then plated into **colony formation assays (CFA)** on the second day of isolation.
> Bell et al., page 14, Example 1, paragraph [0121], emphasis added.

> Unless indicated otherwise, **under standard CFA conditions ...**
> Bell et al., page 14, Example 1, paragraph [0121], emphasis added.

> The number of hematopoietic progenitors was scored **between days 13-15 by counting the number and types of colonies present**.
> Bell et al., page 14, Example 1, paragraph [0122], emphasis added.

> Erythroid progenitor **colony formation assays were conducted as described in Example 1 ...**
> Bell et al., page 15, Example 2, paragraph [0124], emphasis added.

Unlike the claimed invention, Bell et al. disclose only the traditional colony forming assay of the prior art. The colony forming assay is a differentiation assay in which cells are allowed to differentiate and form colonies or clusters. Once the cells have differentiated into mature cell types, the colonies can be identified morphologically and counted manually.

The Claimed Invention

The claimed invention provides a proliferation assay for determining the proliferative status of a population of primitive hematopoietic cells. The claimed invention is fundamentally different from any of the cited art, as none of the cited art teaches or even suggests a method for determining the proliferative status of a population of primitive hematopoietic cells.

The present application explains:

> **The high-throughput stem/progenitor cell assay (HT-SPCA) of the present invention does not count colonies or differentiate between colony types. Rather, the HT-SPCA of the present invention measures the proliferation status of cells within the colonies by determining the amount of ATP being produced by the cells**. With

> colony growth in the methyl cellulose assay system of the present invention, some cells in the cultures will begin to proliferate and form aggregates or clusters. However, the proliferative status of the cell population may be limited due to their late stage of differentiation. Thus, a small colony may ensue within a short incubation period, but cell proliferation will rapidly cease.
> Application as filed, page 23, lines 8-16, emphasis added.

> This **ability of the assay method of the present invention to distinguish primitive hematopoietic cells from more mature, differentiated lineages contrasts with the conventional manual assay methods**. **In the manual assay, in which colonies are counted under a microscope, proliferating cells cannot be readily distinguished from non-proliferating cells**. The size of the colony, however, may indicate the "primitiveness" of the cell that gave rise to that colony. Thus, the larger the colony, the greater the possibility of the colony deriving from a more primitive cell. **In the HT-SPCA method of the present invention, the size of the colony of the present invention, however, is irrelevant**. Rather, **it is the proliferative status of the cells within the colonies within the same well that is measured**, as documented in Example 3 below.
> Application as filed, page 23, line 27 - page 24, line 7, emphasis added.

The present application also contains data evidencing some of the differences between the claimed invention and the prior art. Specifically, Example 4, on pages 39-40, and Figures 5 and 6 show that the claimed assay is measuring a different parameter than the colony forming assay found in the prior art. Example 4 compares the data obtained from a colony forming assay with the data obtained from the proliferation assay of the claimed invention.

The discussion in Example 4 explains:

> EXAMPLE 4 : Proliferation of Hematopoietic Stem and Progenitor Cells Measured by Colony Counting and ATP Determination
>
> When cell proliferation was measured as a function of time in culture, some aggregates or colonies contained cells that were proliferating, while others were not, as shown in FIGS. 5A-5C. Wells, therefore, could contain few colonies, but still exhibit high cell proliferation. The results shown in **FIGS. 5A-5C show that the number of cell clusters counted per well does not correlate with the cell proliferation** as detected using the luminescence of the present invention.
>
> In contrast, in those wells in which minimal or no cluster formation was detected, luminescence could be detected. **In some wells, the luminescence was significantly greater than expected from the number of cell clusters counted, indicating that cell proliferation was occurring and that the proliferating cells, were primitive because of their increased proliferative capacity**. On day 10, most wells contained cells that were proliferating. By day 14, this proliferative capacity was only seen in some wells, indicating that proliferation has ceased (RLU lower than the cell cluster count) or is declining. **Those wells exhibiting a significantly greater RLU than determined by manual cell cluster counting showed that cells were present that were capable of extensive proliferation and were probably stem cells**.
>
> Little or no correlation existed between the number of individual colonies and the luminescence, as shown in FIGS. 6A-6C.
> Application as filed, page 39, lines 1-26, emphasis added.

As explained in Example 4 and illustrated in Figure 5, the number of cell clusters counted per well does not correlate with the relative luminescence per well. The reason for the lack of correlation is because the colony forming assay is a differentiation assay, whereas the claimed assay is a proliferation assay.

Figures 5 and 6 illustrate these results graphically as explained in the Brief Description of the Drawings:

> FIGS. 5A-5C illustrate histograms showing the number of cell clusters counted manually per well and the relative luminescence units (RLU) per well at day 7 (FIG. 5A), day 10 (FIG. 5B) and day 14 (FIG. 5C) of incubation.
>
> FIGS. 6A-6C graphically illustrate the lack of correlation between cell cluster counts per well and the relative luminescence units (RLU) per well on day 7 (FIG. 6A), day 10 (FIG. 6B) and day 14 (FIG. 6C) of culture incubation.
> Application as filed, page 9, lines 22-28.

In Figure 5, the solid bars represent the number of manually counted clusters per well under conditions of a traditional colony forming assay. These solid bars show the number of mature differentiated clusters in each well. The hatched bars represent the relative luminescence per well and reflect the level of cell proliferation occurring in each well. These two parameters do not correlate well because one is measuring differentiation and the other is measuring proliferation. Where the hatched bar is much higher than the corresponding solid bar for the same well, this means that relatively few numbers of clusters are producing a relatively high level of luminescence. This is an indication that the cells in that particular well are proliferating at a very high rate and are

likely stem cells. The claimed assay makes it possible to determine the proliferative status of a population of primitive cells and to identify stem cells within that population.

Conclusion

Since the Examiner has failed to established a *prima facia* case of obviousness with respect to independent claim 1, all claims depending from independent claim 1 are, therefore, also nonobvious. As stated in the MPEP:

> To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). ... If an independent claim is nonobvious under 35 U.S.C. 103, then any claim depending therefrom is nonobvious. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988). (MPEP § 2143.03, emphasis added.)

In summary, the combination of cited references would not yield the claimed invention, nor render the claimed invention obvious. For at least these reasons, Applicant respectfully requests that the Examiner withdraw the 35 U.S.C. § 103(a) rejection of the pending claims.

The art of record is directed to standard tissue culture media and traditional colony forming assays. Traditional colony-forming assays measure the ability of a primitive cell to differentiate into colonies, and are, therefore, differentiation assays. The present invention is directed to a high-throughput assay method for rapidly determining the proliferative status of a population of primitive hematopoietic cells. In contrast to the colony forming assays, or differentiation assays of the past, the present invention provides a proliferation assay. None of the cited art provides the motivation, suggestion, or teaching for a high-throughput proliferation assay for primitive hematopoietic cells.

Any amendments made during the prosecution of this application are intended solely to expedite prosecution of the application and are not to be interpreted as acknowledgement of the validity of any rejection raised earlier in prosecution, nor as

acknowledgement that any citation made against the application is material to the patentability of the application prior to amendment.

This Amendment and Response is being filed with a Request for Extension of Time for two (2) months under 37 C.F.R. § 1.136(a)(1) and the appropriate fee set forth in 37 C.F.R. § 1.17(a). No additional fees are believed necessitated by the filing of this Amendment and Response. Should any such additional fees be required, the Director is hereby authorized to deduct them from Deposit Account No. 18-2000, of which the undersigned is an authorized signatory.

Should the Examiner believe that there are any outstanding matters capable of resolution by a telephone interview, the Examiner is encouraged to telephone the undersigned attorney of record. Finally, the Applicant and the Applicant's Representative again express their appreciation for the Examiner's time and consideration during the personal interview on 21 August 2007.

Respectfully submitted

Donna E. Becker
Reg. No. 44,529

29 August 2007

ROSENBAUM & ASSOCIATES, P.C.
650 Dundee Road
Suite #380
Northbrook, Illinois 60062
Tel. (847) 770-6000
Fax. (847) 770-6006

Docket No. 6115-007